Human CD8+ T cells release extracellular traps that deliver cytotoxic vesicles to distant cells: a new mechanism of tissue pathology in human tegumentary leishmaniasis?

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Cell death plays an integral role in cellular immunity and is critical for both protective and pathogenic immune responses. Etosis is a cell death mechanism defined by the release of extracellular traps (ETs) composed mainly of DNA and histones. While the release of ETs has been primarily associated with innate immune cells, it has been suggested that B and T lymphocytes might also employ this mechanism. ETs are capable of triggering or amplifying inflammatory responses, and play an important role in the control of infections, since they are capable of physically retaining and eliminating pathogens. These activities suggest an important role for ETs in parasitic infections, both in inducing inflammation as well as controlling the parasite. In fact, it has been demonstrated that Leishmania amazonensis and Trypanosoma cruzi can induce the release of ETs by neutrophils. Human infection with Leishmania braziliensis leads to a series of clinically distinct manifestations of American tegumentary leishmaniasis (LTA), where the cutaneous and mucosal clinical forms are most prevalent in Brazil. These are debilitating and mutilating diseases and, while treatment is available, it presents undesirable side effects. In addition, therapeutic failure has also been a growing issue in endemic areas. Thus, understanding the mechanisms that mediate tissue pathology in LTA may provide targets for alternative therapeutic intervention. We have previously shown that the inflammatory infiltrate present in lesions of patients with LTA caused by the infection with Leishmania braziliensis is primarily composed of CD4+ and CD8+ T cells, and that the tissue pathology in LTA is strongly associated with the presence of CD8+ T cells that express cytotoxic granules. Interestingly, confocal analysis of lesions from LTA patients stained with 4',6-Diamidine-2'-phenylindole dihydrochloride (DAPI), a DNA marker, showed significant staining outside of cells. Given the presence of extracellular DNA in lesions from LTA patients, together with the fact that such lesions are predominantly composed of T cells, and that cytotoxic CD8+ T cells are associated with tissue pathology in LTA, we hypothesized that human T cells are capable of releasing ETs, and that ETs are indeed present in lesions from LTA. To pursue these hypotheses, we used confocal and electron microscopy, as well as biochemical tests and multiparameter flow cytometry analysis to demonstrate that purified human T cells can release ETs after in vitro activation. In addition, we evaluated the occurrence of ETs in lesions from LTA patients using confocal microscopy and double staining with DAPI and anti-histone antibodies, which are, together, characteristics of ETs. Our data unequivocally demonstrated, for the first time, that human CD4+ and CD8+ T cells can release morphologically and functionally distinct ETs, which we named lymphocyte-derived extracellular traps (LETs). Critically, CD8+ T cell-derived LETs form strands that connect distant cells, and co-localize with CD107a, a marker of vesicles containing cytotoxic granules, leading to death of the target cell. In addition, we demonstrated the occurrence of ETs in lesions from LTA patients, with an inflammatory infiltrate primarily composed of T cells, and enriched in cytotoxic CD8+ T cells, suggesting that these structures are LETs. Our findings that CD8+ T cells can release LETs that carry cytotoxic vesicles to distant cells provide a novel contribution towards understanding the mechanisms of CD8-mediated cell death. In addition, we propose that the release of LETs may represent a mechanism related to pathology of human LTA, opening new perspectives for future interventions.